receptor's strikingly different liganding mechanism.

L8 ANSWER 22 OF 79 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 97160642 MEDLINE
DOCUMENT NUMBER: 97160642 PubMed ID: 9006976
TITLE: The **intracellular loop** between domains I and II of the B-type calcium channel confers aspects of **G-protein** sensitivity to the E-type calcium channel.
AUTHOR: Page K M; Stephens G J; Berrow N S; Dolphin A C
CORPORATE SOURCE: Department of Pharmacology, Royal Free Hospital School of Medicine, London NW3 2PF, United Kingdom.
SOURCE: JOURNAL OF NEUROSCIENCE, **(1997 Feb 15)** 17 (4) 1330-8.
Journal code: JDF; 8102140. ISSN: 0270-6474.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970321
Last Updated on STN: 20000303
Entered Medline: 19970310

AB Neuronal voltage-dependent calcium channels undergo inhibitory modulation by **G-protein** activation, generally involving both kinetic slowing and steady-state inhibition. We have shown previously that the beta-subunit of neuronal calcium channels plays an important role in this process, because when it is absent, greater receptor-mediated inhibition is observed (). We therefore hypothesized that the calcium channel beta-subunits normally may occlude **G-protein** -mediated inhibition. Calcium channel beta-subunits bind to the **cytoplasmic loop** between transmembrane domains I and II of the alpha1-subunits (). We have examined the hypothesis that this loop is involved in **G-protein**-mediated inhibition by making **chimeras** containing the I-II loop of alpha1B or alpha1A inserted into alpha1E (alpha1EBE and alpha1EAE, respectively). This strategy was adopted because alpha1B (the molecular counterpart of N-type channels) and, to a lesser extent, alpha1A (P/Q-type) are **G-protein**-modulated, whereas this has not been observed to any great extent for alpha1E. Although alpha1B, coexpressed with alpha2-delta and beta1b transiently expressed in COS-7 cells, showed both kinetic slowing and steady-state inhibition when recorded with GTPgammaS in the patch pipette, both of which were reversed with a depolarizing prepulse, the **chimera** alpha1EBE (and, to a smaller extent, alpha1EAE) showed only kinetic slowing in the presence of GTPgammaS, and this also was reversed by a depolarizing prepulse. These results indicate that the I-II loop may be the molecular substrate of kinetic slowing but that the steady-state inhibition shown by alpha1B may involve a separate site on this calcium channel.

L8 ANSWER 23 OF 79 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 97178816 MEDLINE
DOCUMENT NUMBER: 97178816 PubMed ID: 9063879
TITLE: Ca2+-dependent inhibition of **G protein** -coupled receptor kinase 2 by calmodulin.
AUTHOR: Haga K; Tsuga H; Haga T
CORPORATE SOURCE: Department of Biochemistry, Institute for Brain Research, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Japan.. haga@m.u-tokyo.ac.jp
SOURCE: BIOCHEMISTRY, **(1997 Feb 11)** 36 (6) 1315-21.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970327
Last Updated on STN: 19980206

ered Medline: 19970317

AB Agonist- or lig dependent phosphorylation of m arinic acetylcholine receptor m2 subtypes (m2 receptors) or rhodopsin by **G protein**-coupled receptor kinase 2 (GRK2) was found to be inhibited by calmodulin in a Ca2+-dependent manner. The phosphorylation was fully inhibited in the absence of **G protein** betagamma subunits and partially inhibited in the presence of betagamma subunits. The dose-response curve for stimulation by betagamma subunits of the m2 and rhodopsin phosphorylation was shifted to the higher concentration of betagamma subunits by addition of Ca2+-calmodulin. The phosphorylation by GRK2 of a glutathione S-transferase **fusion** protein containing a peptide corresponding to the central part of the third **intracellular loop** of m2 receptors (I3-GST) was not affected by Ca2+-calmodulin in the presence or absence of betagamma subunits, but the agonist-dependent stimulation of I3-GST phosphorylation by an I3-deleted m2 receptor mutant in the presence of betagamma subunits was suppressed by Ca2+-calmodulin. These results indicate that Ca2+-calmodulin does not directly interact with the catalytic site of GRK2 but inhibits the kinase activity of GRK2 by interfering with the activation of GRK2 by agonist-bound m2 receptors and **G protein** betagamma subunits. In agreement with the assumption that GRK2 activity is suppressed by the increase in intracellular Ca2+, the sequestration of m2 receptors expressed in Chinese hamster ovary cells was found to be attenuated by the treatment with a Ca2+ ionophore, A23187.

L8 ANSWER 24 OF 79 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1997:527737 BIOSIS
DOCUMENT NUMBER: PREV199799826940
TITLE: Modulation of the voltage-dependent calcium channel I-II loop **chimera** alpha-1EBE by G-beta-gamma is not identical to that of alpha-1B.
AUTHOR(S): Berrow, N. S.; Stephens, G. J.; Page, K. M.; Dolphin, A. C.
CORPORATE SOURCE: Dep. Pharmacol., University Coll. RFHSM, London WC1 UK
SOURCE: Society for Neuroscience Abstracts, (1997) Vol. 23, No. 1-2, pp. 1192.
Meeting Info.: 27th Annual Meeting of the Society for Neuroscience New Orleans, Louisiana, USA October 25-30, 1997
ISSN: 0190-5295.
DOCUMENT TYPE: Conference; Abstract; Conference
LANGUAGE: English

L8 ANSWER 25 OF 79 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 97367088 MEDLINE
DOCUMENT NUMBER: 97367088 PubMed ID: 9223879
TITLE: Adrenergic receptor and alpha 2 agonist--2: Structure-function relationship of adrenoceptors.
AUTHOR: Mizobe T
CORPORATE SOURCE: Department of Anesthesiology, Kyoto Prefectural University of Medicine.
SOURCE: MASUI. JAPANESE JOURNAL OF ANESTHESIOLOGY, **(1997 Jun)** 46 (6) 770-6. Ref: 14
Journal code: KHR; 0413707. ISSN: 0021-4892.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970926
Last Updated on STN: 20000303
Entered Medline: 19970912

AB Recombinant DNA experiments using **chimeric** receptors containing portions of alpha 2 and beta 2 adrenoceptors demonstrated structure-function relationships of adrenoceptors. The seventh

transmembrane domain determines the subtype ligand binding specificity between alpha 2 and beta 2 adrenoceptors. A further investigation by mutagenesis suggests that a direct interaction between subtype specific ligands and specific amino acids such as Phe (412) and Asn (312) in the seventh transmembrane domain of the alpha 2 and beta 2 adrenoceptors respectively. The third **cytoplasmic loop** is responsible for determining the specificity of interactions between the receptor and **G protein**. Recombinant DNA technology also demonstrated that **seven transmembrane** domains of adrenoceptors have a counterclockwise arrangement when viewed from the outside of the cell.

L8 ANSWER 26 OF 79 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 97415810 MEDLINE
DOCUMENT NUMBER: 97415810 PubMed ID: 9268722
TITLE: **Chimeric** D2/D3 dopamine receptor coupling to adenylyl cyclase.
AUTHOR: Lachowicz J E; Sibley D R
CORPORATE SOURCE: Experimental Therapeutics Branch, National Institutes of Health, Bethesda, Maryland, 20892-1406, USA.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, **(1997 Aug 18)** 237 (2) 394-9.
Journal code: 9Y8; 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970926
Last Updated on STN: 19980206
Entered Medline: 19970915

AB We have sought to determine which area of the D2 dopamine receptor's third **intracellular loop** contributes to **G-protein** coupling by constructing reciprocal **chimeric** D2/D3 receptors with **fusion** points near the center of the third **intracellular loop**. Both receptor **chimeras** were expressed equally well in Chinese Hamster Ovary (CHO) cells and exhibited ligand binding properties similar to those of the wild type receptors. Surprisingly, both of the D2/D3 receptor **chimeras** were able to effectively inhibit adenylyl cyclase activity to almost the same extent as that seen with the D2 receptor whereas the D3 receptor was without effect. These results suggest that the D2 receptor possesses two redundant and independent domains for **G-protein** coupling and inhibition of adenylyl cyclase activity.

L8 ANSWER 27 OF 79 MEDLINE DUPLICATE 19
ACCESSION NUMBER: 97150731 MEDLINE
DOCUMENT NUMBER: 97150731 PubMed ID: 8995267
TITLE: Identification of A2a adenosine receptor domains involved in selective coupling to Gs. Analysis of **chimeric** A1/A2a adenosine receptors.
AUTHOR: Olah M E
CORPORATE SOURCE: Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710, USA.
CONTRACT NUMBER: P50HL54314 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1997 Jan 3)** 272 (1) 337-44.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970305
Last Updated on STN: 20000303
Entered Medline: 19970218

AB Responses to adenosine are governed by selective activation of distinct G proteins by adenosine receptor (AR) subtypes. The A2aAR couples via Gs to

adenylyl cyclase stimulation while the A1AR couples to Gi to inhibit adenylyl cyclase. To determine regions of the A2aAR that selectively couple to Gs, **chimeric** A1/A2aARs were expressed in Chinese hamster ovary cells and ligand binding and adenylyl cyclase activity analyzed. Replacement of the third **intracellular loop** of the A2aAR with that of the A1AR reduced maximal adenylyl cyclase stimulation and decreased agonist potency. Restricted **chimeras** indicated that the NH2-terminal portion of **intracellular loop** 3 was predominantly responsible for this impairment. Reciprocal **chimeras** composed primarily of A1AR sequence with limited A2aAR sequence substitution stimulated adenylyl cyclase and thus supported these findings. A lysine and glutamic acid residue were identified as necessary for efficient A2aAR-Gs coupling. Analysis of **chimeric** receptors in which sequence of **intracellular loop** 2 was substituted indicated that the nature of amino acids in this domain may indirectly modulate A2aAR-Gs coupling. Replacement of the cytoplasmic tail of the A2aAR with the A1AR tail did not affect adenylyl cyclase stimulation. Thus, selective activation of Gs is predominantly dictated by the NH2-terminal segment of the third **intracellular loop** of the A2aAR.

L8 ANSWER 28 OF 79 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:4470 BIOSIS
DOCUMENT NUMBER: PREV199800004470
TITLE: Binding of multiple ligands to Pleckstrin homology domain regulates membrane translocation and enzyme activity of beta-adrenergic receptor kinase.
AUTHOR(S): Touhara, Kazushige (1)
CORPORATE SOURCE: (1) Dep. Neurochem., Fac. Med., Univ. Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113 Japan
SOURCE: FEBS Letters, (**Nov. 10, 1997**) Vol. 417, No. 2, pp. 243-248.
ISSN: 0014-5793.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Pleckstrin homology (PH) domains are discrete structural modules present in numerous proteins involved in signal transduction processes. In the case of the beta-adrenergic receptor kinase (betaARK), PH domain-mediated binding of two ligands, the betagamma subunits of heterotrimeric G proteins (Gbetagamma) and phosphatidylinositol 4,5-bisphosphate (PIP2), has been shown to be required for the kinase function. In this study, the ability of Gbetagamma and PIP2 to affect membrane localization of betaARK is used to define the ligand binding characteristics of the betaARK PH domain. The binding of these ligands to the PH domain of the intact kinase is shown to be cooperative, Gbetagamma increasing the affinity of the PH domain for PIP2. Notably, although PIP2-dependent membrane association of betaARK is observed at high concentrations of this lipid, in the absence of Gbetagamma, no receptor phosphorylation is observed. Peptides derived from the receptor **intracellular loop** inhibit the receptor phosphorylation without affecting the membrane translocation of the kinase complex, suggesting that betaARK activity does not necessarily correlate with the amount of betaARK associated with the membrane. These results point to a distinct role for each PH domain ligand in betaARK-mediated receptor phosphorylation. Strikingly, the ligand binding characteristics of the isolated betaARK PH domain **fused** to glutathione S-transferase are very different from those of the PH domain of the intact kinase. Thus, in contrast to the native protein, the isolated PH domain binds Gbetagamma and PIP2 independently and with no apparent cooperativity. That protein environment plays an important role in determining the ligand binding characteristics of a particular PH domain highlights the potential risks of inferring mechanisms from studies of isolated PH domains.

L8 ANSWER 29 OF 79 MEDLINE DUPLICATE 20
ACCESSION NUMBER: 1999172472 MEDLINE
DOCUMENT NUMBER: 99172472 PubMed ID: 10072934
TITLE: **Chimeric** dopamine D2/angiotensin AT1 receptors:

[redacted]e of the length of third **intra[redacted]llular loo[redacted]** of D2 receptors in conferring [redacted]cificity of receptor binding and **G-protein** coupling.

AUTHOR: Chen H; Zhang Y Y; Han Q D

CORPORATE SOURCE: Institute of Vascular Medicine, Third Hospital, Beijing Medical University, China.

SOURCE: CHUNG-KUO YAO LI HSUEH PAO [ACTA PHARMACOLOGICA SINICA], **(1997 May)** 18 (3) 209-13.
Journal code: 1P9; 8100330. ISSN: 0253-9756.

PUB. COUNTRY: China
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199904

ENTRY DATE: Entered STN: 19990511
Last Updated on STN: 20000303
Entered Medline: 19990427

AB AIM: To define roles of the third **intracellular loop** (IL3) length of **G-protein** coupled receptors in conferring the specificity for receptor binding and **G-protein** coupling. METHODS: By polymerase chain reaction (PCR), the IL3 of D2 receptor was replaced with the counter part of AT1 receptor which has the shortest loop among all **G-protein** coupled receptors. D2/AT1 receptor cDNA was then stably transfected into Chinese hamster ovary cells and a clone with high level expression was obtained for receptor binding and agonist-induced phosphatidylinositols (PI) turnover experiments. RESULTS: Comparing to the D2 receptor, D2/AT1 **chimeric** receptor had lower affinities for all D2 receptor antagonists tested (spiperone, haloperidol, (+)-butaclamol, chlopromazine, clozapine, trifluoperdazine) and different affinity profiles to agonists (apomorphine, dopamine, quinpirole, bromocriptine). But the **chimeric** receptor failed to couple to **G-protein** and subsequent stimulation of PI turnover. CONCLUSION: The length of IL3 of D2 receptor participates defining recpetor binding sites conformation, and structure beyond IL3 may affect receptor **G-protein** coupling.

L8 ANSWER 30 OF 79 MEDLINE DUPLICATE 21

ACCESSION NUMBER: 1998010463 MEDLINE

DOCUMENT NUMBER: 98010463 PubMed ID: 9350980

TITLE: The C-terminal domain of the human EP4 receptor confers agonist-induced receptor desensitization in a receptor hybrid with the rat EP3beta receptor.

AUTHOR: Neuschafer-Rube F; Hanecke K; Puschel G P

CORPORATE SOURCE: Institut fur Biochemie und Molekulare Zellbiologie, Gottingen, Germany.

SOURCE: FEBS LETTERS, **(1997 Sep 29)** 415 (2) 119-24.
Journal code: EUH; 0155157. ISSN: 0014-5793.

PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199711

ENTRY DATE: Entered STN: 19980109
Last Updated on STN: 20000303
Entered Medline: 19971128

AB Prostaglandin E2 receptors (EPR), which belong to the family of heterotrimeric **G protein**-coupled ectoreceptors with **seven transmembrane** domains, can be classified into four subtypes according to their ligand binding and **G protein** coupling specificity. Of these, EP3betaR is coupled to Gi, whereas EP4R is coupled to Gs. EP4R, in contrast to EP3betaR, shows agonist-induced desensitization. The C-terminal domain and the third **intracellular loop** of these receptors have been implicated in **G protein** coupling specificity and desensitization. Here, receptor hybrids consisting of the main portion of rat EP3betaR and either the

C-terminal domain or the third **intracellular loop** of human EP4R were used to study the contribution of the respective receptor domains to **G protein** coupling and desensitization. Neither the EP4R C-terminal domain nor the EP4R third **intracellular loop** alone was sufficient to change the coupling specificity of the rEP3hEP4 receptor hybrids from Gi to Gs or to confer additional Gs coupling. However, the EP4R C-terminal domain but not the third **intracellular loop** was necessary and sufficient to mediate rapid agonist-induced, second messenger-independent desensitization in the Gi-coupled hybrid receptors.

L8 ANSWER 31 OF 79 MEDLINE DUPLICATE 22
ACCESSION NUMBER: 97094866 MEDLINE
DOCUMENT NUMBER: 97094866 PubMed ID: 8940100
TITLE: Functional role of the third **cytoplasmic loop** in muscarinic receptor dimerization.
AUTHOR: Maggio R; Barbier P; Fornai F; Corsini G U
CORPORATE SOURCE: Institute of Pharmacology, School of Medicine, University of Pisa, Via Roma 55, 56100 Pisa, Italy.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 Dec 6)** 271 (49) 31055-60.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19980206
Entered Medline: 19970109

AB By means of the expression of two **chimeric** receptors, alpha2/m3 and m3/alpha2, in which the carboxyl-terminal receptor portions, containing transmembrane (TM) domains VI and VII, were exchanged between the alpha2C adrenergic and the m3 muscarinic receptor, Maggio et al. (Maggio, R., Vogel, Z., and Wess, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3103-31073) demonstrated that **G protein**-linked receptors are able to interact functionally with each other at the molecular level to form (hetero)dimers. In the present study we tested the hypothesis that interaction between receptors might depend on the presence of a long third intracellular (i3) loop and that shortening this loop could impair the capability of receptors to form dimers. To address this question, we initially created short **chimeric** alpha2 adrenergic/m3 muscarinic receptors in which 196 amino acids were deleted from the i3 loop (alpha2/m3-short and m3/alpha2-short). Although co-transfection of alpha2/m3 and m3/alpha2 resulted in the appearance of specific binding, the co-expression of the two short constructs (alpha2/m3-short and m3/alpha2-short), either together or in combination, respectively, with m3/alpha2 and alpha2/m3 did not result in any detectable binding activity. In another set of experiments, a mutant m3 receptor, m3/m2(16aa), containing 16 amino acids of the m2 receptor sequence at the amino terminus of the third **cytoplasmic loop**, which was capable of binding muscarinic ligands but was virtually unable to stimulate phosphatidylinositol hydrolysis, was also mutated in the i3 loop, resulting in the m3/m2(16aa)-short receptor. Although co-transfection of m3/m2(16aa) with a truncated form of the m3 receptor (m3-trunc, containing an in frame stop codon after amino acid codon 272 of the rat m3 sequence) resulted in a considerable carbachol-stimulated phosphatidylinositol breakdown, the co-transfection of m3/m2(16aa)-short with the truncated form of the m3 receptor did not result in any recovery of the functional activity. Thus, these data suggest that intermolecular interaction between muscarinic receptors, involving the exchange of amino-terminal (containing TM domains I-V) and carboxyl-terminal (containing TM domains VI and VII) receptor fragments depends on the presence of a long i3 loop. One may speculate that when alternative forms of receptors with a different length of the i3 loop exist, they could have a different propensity to dimerize.

L8 ANSWER 32 OF 79 MEDLINE DUPLICATE 23
ACCESSION NUMBER: 97067128 MEDLINE
DOCUMENT NUMBER: 97067128 PubMed ID: 8910530
TITLE: Membrane targeting and determination of transmembrane topology of the human vasopressin V2 receptor.
AUTHOR: Schulein R; Rutz C; Rosenthal W
CORPORATE SOURCE: Rudolf-Buchheim-Institut fur Pharmakologie, Justus-Liebig-Universitat Giessen, Frankfurter Strasse 107, D-35392 Giessen, Germany.. Walter.Rosenthal@pharma.med.uni-giessen.de
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 Nov 15)** 271 (46) 28844-52.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19970107

AB The human vasopressin V2 receptor belongs to the large family of **G-protein**-coupled receptors, which possess **seven transmembrane** helices, an extracellular N terminus and an intracellular C terminus. We have determined the sequence requirements of the V2 receptor for membrane insertion and correct topology for the inner membrane of Escherichia coli with the PhoA/LacZ gene **fusion** system. In addition, we have studied the signals for its membrane insertion and correct topology for the membrane of the endoplasmic reticulum of the authentic eucaryotic transport system. To this end, we have extended the PhoA/LacZ gene **fusion** system for the first time to eucaryotic cells, i.e. transiently transfected COS.M6 cells. Truncated V2 receptor sequences were **fused** to PhoA and LacZ and expressed in both E. coli and COS.M6 cells. Cells were fractionated, and LacZ/PhoA activity assays and immunoblots were performed. We show here that a V2 receptor fragment consisting of the N terminus, the first transmembrane segment and the first **cytoplasmic loop** (71 amino acids) provided sufficient information for membrane insertion and correct orientation (extracellular N terminus) in both procaryotic and eucaryotic cells. Our data differ substantially from those obtained for the human beta2-adrenergic receptor expressed in E. coli (Lacatena, R. M., Cellini, A., Scavizzi, F., and Tocchini-Valentini, G. P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10521-10525). To establish correct topology, the beta2-adrenergic receptor requires a larger receptor portion, including the three N-terminal transmembrane segments and/or parts of the second **cytoplasmic loop**. The present data show that the observations made for the beta2-adrenergic receptor cannot be applied to **G-protein**-coupled receptors generally.

L8 ANSWER 33 OF 79 MEDLINE DUPLICATE 24
ACCESSION NUMBER: 96394622 MEDLINE
DOCUMENT NUMBER: 96394622 PubMed ID: 8798728
TITLE: The role of charged residues in determining transmembrane protein insertion orientation in yeast.
AUTHOR: Harley C A; Tipper D J
CORPORATE SOURCE: Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, Massachusetts 01655, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 Oct 4)** 271 (40) 24625-33.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: lish
FILE SEGMENT: ority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961125

AB The first 79 residues of the yeast Ste2p **G protein** -coupled pheromone receptor, including the negatively charged N-terminal domain, the first transmembrane segment, and the following positively charged **cytoplasmic loop**, has been **fused** to a Kex2p-cleavable beta-lactamase reporter. Insertion orientation was determined by analysis of cell-associated and secreted beta-lactamase activities and independently corroborated by analysis of membrane association and glycosylation patterns. This **fusion** inserts with exclusively N terminus exofacial (Nexo) topology, serving as a model type III membrane protein. Orientation is unaffected by removal of all three positively charged residues in the **cytoplasmic loop** or by deletion of all but 12 residues from the N-terminal domain. The residual -2 N-terminal charge apparently provides a signal sufficient to determine Nexo topology. This is entirely consistent with the statistically derived rule in which the charge difference, Delta(C-N), counted for the 15 immediately flanking residues, is the primary topology determinant. Mutations altering Delta(C-N) to zero favors Nexo insertion by 3 to 1, whereas increasingly negative values cause increasing inversion of orientation. All results are consistent with the charge difference rule and indicate that whereas positive charges promote cytoplasmic retention, negative charges promote translocation.

L8 ANSWER 34 OF 79 MEDLINE DUPLICATE 25
ACCESSION NUMBER: 96394402 MEDLINE
DOCUMENT NUMBER: 96394402 PubMed ID: 8798508
TITLE: The third intracellular domain of the platelet-activating factor receptor is a critical determinant in receptor coupling to phosphoinositide phospholipase C-activating G proteins. Studies using intracellular domain minigenes and receptor **chimeras**.
AUTHOR: Carlson S A; Chatterjee T K; Fisher R A
CORPORATE SOURCE: Department of Pharmacology, University of Iowa College of Medicine, Iowa City, Iowa 52242, USA.
CONTRACT NUMBER: DK25295 (NIDDK)
HL41071 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 Sep 20)** 271 (38) 23146-53.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 20000303
Entered Medline: 19961119

AB Platelet activating factor (PAF) is a potent phospholipid mediator which elicits a diverse array of biological actions by interacting with **G protein**-coupled PAF receptors (PAFR). Binding of PAF to PAFRs leads to activation of **G protein**(s) that stimulate phosphoinositide phospholipase C and subsequent intracellular signaling responses. To identify the potential role of intracellular domains of the rat PAFR (rPAFR) in signaling, we examined effects of transfecting minigenes encompassing rPAFR intracellular domains 1 (1i), 2 (2i), and 3 (3i) on inositol phosphate (IP) production mediated by the co-transfected rPAFR cDNA. Although transfection of the rPAFR1i and rPAFR2i minigenes had no effects on PAF-stimulated signaling, transfection of the rPAFR3i minigene inhibited PAF-stimulated IP production by approximately 50% compared to controls. The rPAFR3i domain did not inhibit

IP production mediated by the multifunctional rat pituitary adenylate cyclase-activating polypeptide receptor (rPACAPR) demonstrating the specificity of the competition by the rPAFR3i domain. In further experiments, the rPAFR3i domain was engineered onto the homologous domain of a monofunctional transmembrane variant of the rPACAPR (rPACAPR2) that activates only adenylyl cyclase. The rPACAPR2/rPAFR3i **chimera** responded to PACAP with increases in IP production which were attenuated nearly completely in cells cotransfected with the rPAFR3i domain. In contrast, PACAP had no effects on IP production in a receptor **chimera** expressing a mutated form of the rPAFR3i domain (rPACAPR2/rPAFR3imut). These results demonstrate the ability of the rPAFR3i domain to confer a phospholipase C-signaling phenotype to a receptor deficient in this activity and show that this activity is specific for the engineered rPAFR3i domain. These results suggest that the third **intracellular loop** of the rPAFR is a primary determinant in its coupling to phosphoinositide phospholipase C-activating G proteins, providing the first insight into the molecular basis of interaction of PAFRs with signal-transducing G proteins.

L8 ANSWER 35 OF 79 MEDLINE DUPLICATE 26
ACCESSION NUMBER: 96394306 MEDLINE
DOCUMENT NUMBER: 96394306 PubMed ID: 8798412
TITLE: Transmembrane regions V and VI of the human luteinizing hormone receptor are required for constitutive activation by a mutation in the third **intracellular loop**.
AUTHOR: Kudo M; Osuga Y; Kobilka B K; Hsueh A J
CORPORATE SOURCE: Division of Reproductive Biology, the Department of Obstetrics and Gynecology, Stanford University Medical School, Stanford, California 94305, USA.
CONTRACT NUMBER: HD-23273 (NICHD)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 Sep 13)** 271 (37) 22470-8.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961107

AB Gonadotropin receptors are members of the **seven-transmembrane** (TM) receptor family. Several point mutations in TM V and VI and the **intracellular loop** 3 (i3) have been identified in the luteinizing hormone (LH) receptor gene, leading to constitutive activation of the receptor. Because gonadotropin receptors are highly conserved, we mutated the follicle-stimulating hormone (FSH) receptor at the corresponding amino acids. However, the FSH receptor mutants showed minimal increases in basal cAMP production. Taking advantage of this difference between the two receptors, we designed **chimeric** receptors with or without a point mutation in the i3 to identify the region in the LH receptor important for its constitutive activation. Introduction of the point mutation into **chimeric** receptors containing only TM V to VI from the LH receptor led to major increases in ligand-independent cAMP production. Furthermore, a **chimeric** receptor with only TM V and VI derived from the LH receptor can be rendered constitutively active by the mutation in the i3 from the FSH receptor. These results suggest that interactions between TM V and VI of the FSH receptor are essential for maintaining the receptor in the more constrained state, whereas interactions between these domains of the LH receptor are permissive for constitutively activating mutations in the i3.

L8 ANSWER 36 OF 79 MEDLINE DUPLICATE 27
ACCESSION NUMBER: 96355570 MEDLINE

DOCUMENT NUMBER: [redacted]55570 PubMed ID: 8702980
TITLE: [redacted]ferential regulation of **G-prot**[redacted]
-mediated signaling by chemokine receptors.
AUTHOR: Arai H; Charo I F
CORPORATE SOURCE: Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, California 94141-9100, USA.
CONTRACT NUMBER: HL52773 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 Sep 6)** 271 (36) 21814-9.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961022
Last Updated on STN: 20000303
Entered Medline: 19961010

AB Monocyte chemoattractant protein-1 (MCP-1) is a member of a family of chemotactic cytokines that induce directed migration of leukocytes via activation of **seven-transmembrane** domain receptors. To identify G-proteins that couple to the two forms of the MCP-1 receptor, as well as to related chemokine receptors, we have performed cotransfection experiments in mammalian cells. In COS-7 cells, the type A and type B MCP-1 receptors coupled to Galphai, Galphaq, and Galpha16, whereas the macrophage inflammatory protein-1alpha/RANTES (regulated on activation, normal T cell-expressed and secreted) receptor (C-CR1) coupled to Galphai and Galphaq but failed to couple to Galpha16. In HEK-293 cells, however, the MCP-1 receptors and C-CR1 coupled to Galphaq but failed to couple to Galpha16. In contrast, the interleukin-8 and C5a receptors did not couple to Galphaq in either COS-7 or HEK-293 cells but did couple to Galpha16. Exchange of **intracellular loops** between the MCP-1 and interleukin-8 receptors to create **chimeric** receptors revealed that the third loop of the MCP-1 receptor accounted for virtually all of the coupling to Galphaq. We conclude that the MCP-1 and related chemokine receptors couple to multiple G-proteins, that coupling is cell type-specific, and that the third **intracellular loop** of the C-C type receptors mediates Galphaq coupling.

L8 ANSWER 37 OF 79 MEDLINE DUPLICATE 28
ACCESSION NUMBER: 96278671 MEDLINE
DOCUMENT NUMBER: 96278671 PubMed ID: 8662784
TITLE: **Chimeric** mutagenesis of putative **G-protein** coupling domains of the alpha2A-adrenergic receptor. Localization of two redundant and fully competent gi coupling domains.
AUTHOR: Eason M G; Liggett S B
CORPORATE SOURCE: Department of Medicine (Pulmonary), University of Cincinnati College of Medicine, Cincinnati, Ohio 45267, USA.
CONTRACT NUMBER: HL41496 (NHLBI)
HL53436 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 May 31)** 271 (22) 12826-32.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960822
Last Updated on STN: 20000303
Entered Medline: 19960815

AB We have investigated potential Gi and Gs coupling domains within the intracellular regions of the alpha2AAR subtype using a series of nine **chimeric** mutations. The second **intracellular loop** (ICL2, amino acids 133-149) and the amino- and

carboxyl-termi regions of the third **intracell[illegible]r loop** (ICL3, am acids 218-235 and 355-371, res[illegible]tively) of the cloned human alpha2AAR were substituted with the analogous sequence from either the Gs-coupled beta2AR or the Gi-coupled serotonin type 1A receptor (5-HT1AR). Mutant and wild type alpha2AAR were stably expressed in Chinese hamster ovary cells and functional coupling of each receptor to Gi and Gs was assessed in membrane adenylyl cyclase assays. Substitution of 5-HT1AR sequence into ICL2 ablated coupling to Gs but not to Gi, whereas substitution of beta2AR sequence significantly depressed coupling to Gi but not to Gs. Thus, the ICL2 of the alpha2AAR contains elements essential for both signaling pathways. Substitution of either the amino- or carboxyl-terminal segments of ICL3 with 5-HT1AR sequence ablated agonist stimulation of adenylyl cyclase activity (without affecting inhibition), suggesting that both domains are necessary for alpha2AAR coupling to Gs. In contrast, individual substitution of beta2AR sequence into ICL3 amino or carboxyl termini had no appreciable effect on Gi coupling. Concomitant substitution of beta2AR sequence into both regions substantially impaired Gi coupling, implying that each is capable of independently supporting functional coupling. Substitution of 5-HT1AR at either locus had no effect on Gi coupling. Thus, for Gs coupling, these two domains within ICL3 are both required for functional coupling. However, for Gi coupling, the alpha2AAR appears to have two distinct regions within ICL3 that are capable of supporting Gi coupling independently. There has been no previous elucidation of a receptor having redundant, fully competent domains for coupling to a single class of **G-protein**. Such duplicity of functional domains within alpha2AR may suggest strong evolutionary pressure to maintain Gi coupling.

L8 ANSWER 38 OF 79 MEDLINE
ACCESSION NUMBER: 96224088 MEDLINE
DOCUMENT NUMBER: 96224088 PubMed ID: 8621513
TITLE: Different single receptor domains determine the distinct **G protein** coupling profiles of members of the vasopressin receptor family.
AUTHOR: Liu J; Wess J
CORPORATE SOURCE: Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland 20892, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 Apr 12)** 271 (15) 8772-8.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960627
Last Updated on STN: 20000303
Entered Medline: 19960620

AB The vasopressin receptor family is unique among all classes of peptide receptors in that its individual members couple to different subsets of G proteins. The V1a vasopressin receptor, for example, is preferentially linked to G proteins of the Gq/11 class (biochemical response: stimulation of phosphatidylinositol hydrolysis), whereas the V2 vasopressin receptor is selectively coupled to Gs (biochemical response: stimulation of adenylyl cyclase). To elucidate the structural basis underlying this functional heterogeneity, we have systematically exchanged different intracellular domains between the V1a and V2 receptors. Transient expression of the resulting hybrid receptors in COS-7 cells showed that all mutant receptors containing V1a receptor sequence in the second **intracellular loop** were able to activate the phosphatidylinositol pathway with high efficiency. On the other hand, only those hybrid receptors containing V2 receptor sequence in the third **intracellular loop** were capable of efficiently

stimulating cAM[redacted]roduction. These findings sugg[redacted] that the differential **G protein** coupl[redacted] profiles of individual member[redacted]f a structurally closely related receptor subfamily can be determined by different single intracellular receptor domains.

L8 ANSWER 39 OF 79 MEDLINE DUPLICATE 29
ACCESSION NUMBER: 96216503 MEDLINE
DOCUMENT NUMBER: 96216503 PubMed ID: 8632012
TITLE: Identification of a novel receptor kinase that phosphorylates a phospholipase C-linked muscarinic receptor.
AUTHOR: Tobin A B; Keys B; Nahorski S R
CORPORATE SOURCE: Leicester University, Department of Cell Physiology and Pharmacology, P.O. Box 138, Medical Sciences Building, University Road, Leicester, LE1 9HN, United Kingdom.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 Feb 16)** 271 (7) 3907-16.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960715
Last Updated on STN: 20000303
Entered Medline: 19960702

AB Phosphorylation of **G-protein**-linked receptors is thought to play a central role in receptor regulation and desensitization.
Unlike the case of the extensively studied beta-adrenergic receptor/adenylate cyclase pathway, in which receptor-specific phosphorylation is known to be mediated by beta-adrenergic receptor kinase
(beta-ARK), the kinases responsible for phosphorylation of phospholipase C-linked receptors have yet to be identified, although a role for beta-ARK
has been implicated. This study describes the purification of a novel 40-kDa receptor kinase from porcine cerebellum that is able to phosphorylate the phospholipase C-linked m3-muscarinic receptor in an agonist-dependent manner. The assay for kinase activity was based on the ability of the kinase to phosphorylate a bacterial **fusion** protein, Ex-m3, containing amino acids Ser345-Leu463 of the third **intracellular loop** of the m3-muscarinic receptor.
Purification of the muscarinic receptor kinase from a high speed supernatant fraction of porcine cerebellum was achieved using the following steps: (i) 30-60% ammonium sulfate cut and successive chromatography on (ii) butyl-Sepharose (iii) Resource Q, (iv) Resource S, and (v) heparin-Sepharose. The purified protein kinase represented an approximately 18,600-fold purification and was a single polypeptide with a
molecular weight of approximately 40 kDa. Based on the chromatographic mobility, molecular weight, and kinase inhibitor studies, the kinase, designated MRK, was shown to be distinct from previously characterized second messenger regulated protein kinases, beta-ARK, and other members of
the **G-protein**-linked receptor kinase family. It therefore represents a new class of receptor kinase.

L8 ANSWER 40 OF 79 MEDLINE DUPLICATE 30
ACCESSION NUMBER: 96147201 MEDLINE
DOCUMENT NUMBER: 96147201 PubMed ID: 8567679
TITLE: The second **intracellular loop** of metabotropic glutamate receptor 1 cooperates with the other
intracellular domains to control coupling to G-proteins.
AUTHOR: Gomeza J; Joly C; Kuhn R; Knopfel T; Bockaert J; Pin J P
CORPORATE SOURCE: UPR 9023 CNRS-INSERM de Pharmacologie-Endocrinologie, Montpellier, France.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1996 Jan 26)**

(4) 2199-205.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199603
ENTRY DATE: Entered STN: 19960315
Last Updated on STN: 20000303
Entered Medline: 19960301

AB Metabotropic glutamate receptors (mGluR) share no sequence homology with any other **G-protein**-coupled receptors (GPCRs). The characterization of their **G-protein** coupling domains will therefore help define the general rules for receptor-**G-protein** interaction. To this end, the intracellular domains of mGluR3 and mGluR1, receptors coupled negatively to adenylyl cyclase and positively to phospholipase C, respectively, were systematically exchanged. The ability of these **chimeric** receptors to induce Ca2+ signals were examined in Xenopus oocytes and HER 293 cells. The **chimeric** receptors that still possessed the second **intracellular loop** (i2) of these proteins were targeted correctly to the plasma membrane. Consistent Ca2+ signals could be recorded only with **chimeric** mGluR3 receptors that contains i2 and at least one other intracellular domains of mGluR3 have to be replaced by their mGluR1 equivalent to produce optimal coupling to **G protein**. These observations indicate that i2 of mGluR1 is a critical element in determining the transduction mechanism of this receptor. These results suggest that i2 of mGluRs may play a role similar to i3 of most other GPCRs in the specificity of coupling to the G-proteins. Moreover, as in many other GPCRs, our data revealed cooperation between the different mGluR intracellular domains to control efficient coupling to G-proteins.

L8 ANSWER 41 OF 79 MEDLINE DUPLICATE 31
ACCESSION NUMBER: 97039695 MEDLINE
DOCUMENT NUMBER: 97039695 PubMed ID: 8885247
TITLE: The C-terminal third of the human luteinizing hormone (LH) receptor is important for inositol phosphate release: analysis using **chimeric** human LH/follicle-stimulating hormone receptors.
AUTHOR: Hirsch B; Kudo M; Naro F; Conti M; Hsueh A J
CORPORATE SOURCE: Department of Gynecology and Obstetrics, Stanford University School of Medicine, California 94305-5317, USA.
CONTRACT NUMBER: HD-23273 (NICHD)
SOURCE: MOLECULAR ENDOCRINOLOGY, **(1996 Sep)** 10 (9) 1127-37.
Journal code: NGZ; 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970130

AB Gonadotropin and TSH receptors represent a subgroup of **seven transmembrane**-spanning, **G protein**-coupled receptors with a large extracellular ligand-binding region. After ligand binding to their receptors, the majority of actions of gonadotropins and TSH are believed to be mediated by the cAMP-protein kinase A pathway. Although formation of inositol phosphates (IP) has been reported after stimulation of rodent gonadotropin receptors, activation of phospholipase C after ligand binding of human LH or FSH receptors has not been investigated. Human gonadotropin receptors were transiently expressed in 293 cells, and the agonist-induced stimulation of IP formation was measured. The LH receptor responded to a saturating dose of human CG (hCG)

with a 5.2-fold increase of IPs whereas the FSH receptor responded to a saturating dose of FSH with only a 50% increase. On the basis of these differences and in view of the homologous nature of the two gonadotropin receptors, **chimeric** receptors were constructed using domain transfer to identify the regions in the human LH receptor important for phosphatidylinositol hydrolysis. **Chimeric** receptors containing the entire extracellular region of the FSH receptor and the **seven transmembrane** region plus the cytoplasmic tail of the LH receptor responded to FSH treatment with a 4.7-fold increase in IP accumulation. In contrast, the **chimeric** receptor with the extracellular region of the LH receptor and the TM region plus the cytoplasmic tail of the FSH receptor responded minimally (50%) to hCG treatment. When the C-terminal third (from TM V to the cytoplasmic tail) of the FSH receptor was replaced with the LH receptor sequence, the **chimeric** receptor still responded to FSH treatment with a large (6.2-fold) increase in IP release, similar to that of the wild type LH receptor (to hCG), suggesting that C-terminal third of the human LH receptor confers IP signaling ability. This functional domain was further divided into two areas, namely TM V to TM VI and TM VII to the cytoplasmic tail. The **chimeric** receptors F(I-IV)L(V-VI)F(VII-C)R and F(I-VI)L-VII-C)R, in which these two regions of the FSH receptor were replaced by the corresponding sequences of the LH receptor, responded to FSH treatment with partial increases in phosphatidylinositol hydrolysis (2.0- and 3.7-fold, respectively). Furthermore, when TM VII and the cytoplasmic tail of the LH receptor were replaced with the corresponding sequence of the FSH receptor, this **chimeric** receptor showed a diminished (2.0-fold) response to hCG in IP release. For all the **chimeric** receptor constructs analyzed, overall expression, equilibrium binding constants, and adenyl cyclase activation were not altered. Thus, unlike studies using **chimeric** muscarinic and dopaminergic receptors in which the second and third **intracellular loops** were found to be important for IP signaling, the entire C-terminal third of the human LH receptor is important for IP release. Future analysis using the **chimeric** receptor approach should provide new information on the structure-function relationship of gonadotropin, TSH, and other **seven transmembrane**-spanning receptors.

L8 ANSWER 42 OF 79 MEDLINE DUPLICATE 32
ACCESSION NUMBER: 97012669 MEDLINE
DOCUMENT NUMBER: 97012669 PubMed ID: 9157772
TITLE: Phosphorylation of the cytoplasmic tail of the PTH/PTHrP receptor.
AUTHOR: Blind E; Bambino T; Huang Z; Bliziotes M; Nissenson R A
CORPORATE SOURCE: Endocrine Unit, Veterans Affairs Medical Center, University of California, San Francisco, USA.
CONTRACT NUMBER: DK35323 (NIDDK)
SOURCE: JOURNAL OF BONE AND MINERAL RESEARCH, **(1996 May)** 11 (5) 578-86.
Journal code: 130; 8610640. ISSN: 0884-0431.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970602
Last Updated on STN: 19970602
Entered Medline: 19970520

AB Activation of the **G protein**-coupled receptor for parathyroid hormone (PTH)/PTH-related protein (PTHrP) produces homologous desensitization of receptor signaling. We have shown recently that the opossum PTH/PTHrP receptor stably expressed in human embryonic kidney (HEK) 293 cells is phosphorylated upon agonist binding and upon activation of serine/threonine protein kinases (PKA and PKC), an event which for some

G protein-coupl[redacted] receptors has been linked to desensitization. To locate the sites of phospho tion, mutated forms of the opossum PTH/PTHrP receptor were stably expressed in HEK 293 cells, and ligand-stimulated receptor phosphorylation was evaluated. The five serine and threonine residues of the third **cytoplasmic loop** of the receptor were not required for receptor phosphorylation. Basal and ligand-induced phosphorylation were, however, completely abolished upon deletion of all but the 16 juxtamembrane residues of the cytoplasmic C-terminal tail of the receptor, even though this truncated receptor resembled the wild-type receptor in its level of expression based on Western blotting and radioligand binding. To identify further the phosphorylation sites, the 129 amino acid C-terminal tail of the rat PTH/PTHrP receptor was expressed in E. coli as a recombinant glutathione S-transferase **fusion** protein. Elimination of a single PKA consensus site in the tail (serine 491) resulted in > or = 90% loss of PKA-mediated phosphorylation, identifying this as the preferential site for PKA, with two other sites (serine 473 and/or 475) being minor sites. Phosphorylation by PKC occurred largely in the proximal portion of the tail, whereas beta-adrenergic receptor kinase 1 (beta ARK1) phosphorylated more distally in the tail. The ability of these kinases to phosphorylate the PTH/PTHrP receptor at distinct sites on the cytoplasmic tail may allow differential regulation of receptor signaling and trafficking.

L8 ANSWER 43 OF 79 MEDLINE DUPLICATE 33
ACCESSION NUMBER: 95355382 MEDLINE
DOCUMENT NUMBER: 95355382 PubMed ID: 7629092
TITLE: Influence of second and third **cytoplasmic loops** on binding, internalization, and coupling of **chimeric** bombesin/m3 muscarinic receptors.
AUTHOR: Tseng M J; Coon S; Stuenkel E; Struk V; Logsdon C D
CORPORATE SOURCE: Department of Physiology, University of Michigan, Ann Arbor 48109-0622, USA.
CONTRACT NUMBER: DK41350 (NIDDK)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1995 Jul 28)** 270 (30) 17884-91.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 19950921
Last Updated on STN: 19970203
Entered Medline: 19950905

AB In order to investigate the molecular basis for differences in the characteristics of bombesin (Bn) and m3 muscarinic cholinergic (m3 ACh) receptors, **chimeric** Bn receptors possessing cytoplasmic domains from the m3 ACh receptor were produced. The receptors were expressed in CHO-K1 cells and binding, structural, and signal transduction characteristics were analyzed. Cell lines bearing **chimeric** Bn receptors possessing m3 ACh receptor domains in place of either the second **cytoplasmic loop** (BM2L), the third **cytoplasmic loop** (BM3L), or both loops (BM23L) each bound 125I-bombesin with a single affinity that was approximately the same as that of the Bn receptor (5-10 nM). However, Bn receptors possessing the m3 ACh third **cytoplasmic loop** were severely affected in other respects. Internalization of ligand in Bn and BM2L cells was rapid and extensive (> 80% of bound 125I-bombesin was acid-resistant). In contrast, internalization was dramatically reduced in BM3L and BM23L cells (approximately 20% of bound 125I-bombesin was acid-resistant). In Bn or BM2L cells 10 nM bombesin stimulated approximately 10-fold increases in phosphatidylinositol hydrolysis. Activation of Bn receptors also induced an increase in arachidonic acid release (478 +/- 32% of control, n = 3)

and large incr s in intracellular Ca2+. In co rast, in BM3L or BM23L cells, bombesi d no significant effect on ph atidylinositol hydrolysis. Furthermore, BM3L receptor activation did not increase arachidonic acid release. However, BM3L and BM23L cells showed a small increase in intracellular Ca2+ at high concentrations of bombesin. These data indicate that the third **cytoplasmic loop** alone, or together with the second **cytoplasmic loop**, was not sufficient to transfer the characteristics of **G protein** interaction between m3 ACh and bombesin receptors. Furthermore, for the Bn receptor, ligand internalization does, whereas formation of the high affinity binding state does not, appear to require activation of G proteins.

L8 ANSWER 44 OF 79 MEDLINE DUPLICATE 34
ACCESSION NUMBER: 95348089 MEDLINE
DOCUMENT NUMBER: 95348089 PubMed ID: 7622477
TITLE: Identification of a domain in the angiotensin II type 1 receptor determining Gq coupling by the use of receptor **chimeras**.
AUTHOR: Wang C; Jayadev S; Escobedo J A
CORPORATE SOURCE: Cardiovascular Research Institute, University of California, San Francisco 94143-0130, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1995 Jul 14)** 270 (28) 16677-82.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950911
Last Updated on STN: 20000303
Entered Medline: 19950825

AB The angiotensin II type 1 (AT1R) and type 2 (AT2R) receptors belong to the **seven transmembrane** receptor superfamily. Previous studies have suggested that the AT1R couples to a Gq signaling pathway, whereas the AT2R does not associate with Gq. To identify the role that individual intracellular domains play in AT1R function, AT1R/AT2R **chimeric** receptors were prepared by substitution of **intracellular loops**. CHO cells expressing these **chimeras** were used to test angiotensin II-induced c-fos expression and Ca2+ mobilization which are involved in the AT1R signaling pathway through Gq coupling. Substitution of the second **intracellular loop** (IC2) and the cytoplasmic tail between the two receptors did not affect AT1R function. However, exchange of the third **intracellular loop** (IC3) resulted in the loss of function in the AT1R and conferred to the AT2R the ability to constitutively activate the fos promoter. These findings suggest that the third **intracellular loop** of the AT1R is critical for Gq coupling. Substitution of discrete amino acid sequences of the third **intracellular loop** indicate that its N-terminal and C-terminal portions, especially the seven amino acids 219-225 in the N-terminal portion, are important for AT1R function, and that the intermediate portion of this loop is not required for Gq coupling.

L8 ANSWER 45 OF 79 MEDLINE
ACCESSION NUMBER: 96102170 MEDLINE
DOCUMENT NUMBER: 96102170 PubMed ID: 8524820
TITLE: Identification of a receptor/**G-protein** contact site critical for signaling specificity and **G-protein** activation.
AUTHOR: Liu J; Conklin B R; Blin N; Yun J; Wess J
CORPORATE SOURCE: Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Dieseases, Bethesda, MD 20892, USA.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1995 Dec 5)** 92 (25)

642-6.
urnal code: PV3; 7505876. ISSN 027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960219
Last Updated on STN: 20000303
Entered Medline: 19960124

AB Each **G protein**-coupled receptor recognizes only a distinct subset of the many structurally closely related G proteins expressed within a cell. How this selectively is achieved at a molecular level is not well understood, particularly since no specific point-to-point contact sites between a receptor and its cognate **G protein**(s) have been identified. In this study, we demonstrate that a 4-aa epitope on the m2 muscarinic acetylcholine receptor, a prototypical Gi/o-coupled receptor, can specifically recognize the C-terminal 5 aa of alpha subunits of the Gi/o protein family. The m2 receptor residues involved in this interaction are predicted to be located on one side of an alpha-helical receptor region present at the junction between the third **intracellular loop** and the sixth transmembrane domain. Coexpression studies with hybrid m2/m3 muscarinic receptors and mutant **G-protein** alpha q subunits showed that the receptor/**G-protein** contact site identified in this study is essential for coupling specificity and **G-protein** activation.

L8 ANSWER 46 OF 79 MEDLINE DUPLICATE 35
ACCESSION NUMBER: 95247713 MEDLINE
DOCUMENT NUMBER: 95247713 PubMed ID: 7730310
TITLE: Structural basis of **G protein** specificity of human endothelin receptors. A study with endothelinA/B **chimeras**.
AUTHOR: Takagi Y; Ninomiya H; Sakamoto A; Miwa S; Masaki T
CORPORATE SOURCE: Department of Pharmacology, Faculty of Medicine, Kyoto University, Japan.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1995 Apr 28)** 270 (17) 10072-8.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950608
Last Updated on STN: 20000303
Entered Medline: 19950601

AB The endothelin (ET) family of peptides acts via two subtypes of guanine nucleotide-binding regulatory protein (**G protein**)-coupled receptors termed ETA and ETB. ET-1 stimulated cAMP formation in Chinese hamster ovary (CHO) cells stably expressing human wild-type ETA (CHO/hETA cells) while it inhibited cAMP formation in CHO cells expressing human wild-type ETB (CHO/hETB cells), and pharmacological evidence indicated that the opposite effects were due to the selective coupling of each receptor subtype with G alpha s/G alpha i. To find out a receptor domain(s) that determined the selective coupling, a series of **chimeric** receptors between hETA and hETB was expressed on CHO cells, and the effect of ET-1 on cAMP formation in each cell line was tested. hETA with the replacement of second and/or third **intracellular loop** (ICLII and/or -III) to the corresponding region(s) of hETB failed to transmit the stimulatory effect of ET-1. hETB with the replacement of ICLIII to the corresponding region of hETA failed to transmit the inhibitory effect of ET-1. A **chimeric** receptor with ICLII of hETB and with ICLIII of hETA failed to transmit both effects. In cells expressing **chimeric** receptors with ICLII of hETA and with ICLIII of hETB, ET-1 inhibited cAMP

formation whil stimulated cAMP formation whe cells were pretreated with pertussis in. These results indicated th oles of ICLII and -III of hETR as a major determinant of the selective coupling of hETA and hETB with G alpha s/G alpha i, respectively. We also demonstrated that each receptor subtype expressed on the same cell could work independently, i.e.
for hETA to activate G alpha s and for hETB to activate G alpha i, resulting in dose-dependent dual effects of ET-1 on cAMP formation.

L8 ANSWER 47 OF 79 MEDLINE DUPLICATE 36
ACCESSION NUMBER: 95238325 MEDLINE
DOCUMENT NUMBER: 95238325 PubMed ID: 7721739
TITLE: M1 muscarinic receptors heterologously expressed in cardiac
myocytes mediate Ras-dependent changes in gene expression.
AUTHOR: Ramirez M T; Post G R; Sulakhe P V; Brown J H
CORPORATE SOURCE: Department of Pharmacology, University of California at San
Diego, La Jolla 92093, USA.
CONTRACT NUMBER: HL07444 (NHLBI)
HL28143 (NHLBI)
HL46345 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1995 Apr 14)** 270 (15) 8446-51.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950605
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AB Stimulation of alpha 1-adrenergic receptors in neonatal ventricular cardiomyocytes induces hypertrophic changes including activation of the atrial natriuretic factor (ANF) gene. This receptor couples to Gq to activate phospholipase C (PLC) and protein kinase C, which have been implicated as mediators of the hypertrophic response. To directly determine whether receptor coupling to Gq/PLC is sufficient to induce ANF expression, we expressed wild-type and **chimeric** muscarinic cholinergic receptors (mAChRs) with altered **G-protein** coupling properties in cardiac myocytes and examined their ability to activate an ANF promoter/luciferase reporter gene. The cholinergic agonist carbachol failed to induce transcriptional activation of the ANF reporter gene through endogenous Gi-linked M2mAChRs or in cells transfected with M2mAChRs. In contrast, in cells transfected with M1mAChRs, which effectively couple to Gq/PLC, carbachol increased ANF reporter gene expression 10-fold and also increased ANF protein, as determined by immunofluorescence. Carbachol-mediated ANF gene expression was inhibited by the mAChR antagonist pirenzepine with a Ki value characteristic of an M1mAChR. Studies using **chimeric** M1- and M2mAChRs demonstrated that the N-terminal 21 amino acids of the third **intracellular loop** of the M1mAChR were required for receptor coupling to ANF gene expression. This region, previously shown to specify receptor coupling to Gq/PLC, also conferred partial activity to a **chimeric** M2 receptor. We further demonstrated that M1mAChR coupling to ANF gene expression was Ras-dependent since co-expression of dominant-interfering Ala-15 Ras inhibited M1mAChR-induced ANF expression by 60%. In contrast, ANF expression induced by the **chimeric** M2 receptor was not blocked by dominant-interfering Ras. We suggest that receptor coupling to Gq/PLC is sufficient to induce ANF expression and that a Ras-dependent pathway contributes additional signals required for maximal M1mAChR-mediated ANF gene expression.

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TITLE: [redacted]e third **intracellular loop** of t[redacted]
[redacted]hydroxytryptamine-2A receptor [redacted]ermines effector coupling specificity.
AUTHOR(S): Oksenberg, Donna; Havlik, Sona; Peroutka, Stephen J.; Ashkenazi, Avi (1)
CORPORATE SOURCE: (1) Dep. Mol. Biol., Genentech Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080 USA
SOURCE: Journal of Neurochemistry, (1995) Vol. 64, No. 4, pp. 1440-1447.
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LANGUAGE: English

AB 5-Hydroxytryptamine (5-HT) receptors contain seven putative transmembrane domains and couple via different guanine nucleotide binding proteins to specific effector enzymes. Studies with other receptors identify the second and third **intracellular loops** or the C-terminus of the receptor as important for selective effector coupling. However, it is not known which regions of the 5HT receptor determine effector coupling specificity. To address this question, we constructed a **chimeric** 5-HT receptor in which the third intracellular (i3) loop is derived from the 5-HT-2A receptor, which is coupled to activation of phospholipase C, and the rest of the sequence is derived from the 5-HT-1B receptor, which is coupled to inhibition of adenylyl cyclase. The **chimeric** receptor exhibited ligand binding properties similar to those of the 5-HT-1B receptor and distinct from those of the 5-HT-2A receptor. This suggests that the i3 loop is not critical for the unique pharmacology of the 5-HT-1B receptor. In contrast, the **chimeric** receptor exhibited signaling properties similar to those of the 5-HT-2A receptor and distinct from those of the 5-HT-1B receptor. This indicates that the i3 loop determines the effector coupling specificity of the 5-HT-2A receptor.

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AUTHOR: Conquet F
CORPORATE SOURCE: Glaxo Institute for Molecular Biology, Geneva, Switzerland.
SOURCE: NEUROPHARMACOLOGY, **(1995 Aug)** 34 (8) 865-70.
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AB Two main classes of glutamate receptors have been characterized, the ionotropic (iGluRs) and the metabotropic (mGluRs) glutamate receptors. In order to better understand the function of the latter, we have used the technique of gene targeting to generate mice in which the mGluR1 subtype has been inactivated. The disruption was carried out by homologous recombination in embryonic stem (ES) cells at the level of the **seven-membrane** domain, leaving the extracellular part of the receptor untouched. In addition, the reporter gene lacZ was inserted in frame with mGluR1 coding sequence within the second **intracellular loop** of the receptor. The transmission of the mutation to the germ line showed first that the **fusion** protein was functional and second that mGluR1 was inactivated. Therefore, the way homologous recombination was performed in ES cells demonstrated that gene replacement of mGluR1 by lacZ could be a powerful technique to disrupt a gene and at the same time study its endogenous expression.